Immobilized Complexes of Metals with Amino Acid Ligands − A First Step toward the Development of New Biomimetic Catalysts

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The natural amino acids histidine and glutamic acid were immobilized on the surface of functionalized mesoporous silica supports. Histidine and glutamic acid alone and combinations of both were covalently bound to the amino groups on the surface of the support by the formation of amide bonds. The immobilized amino acids were used as ligands for Fe(II). The obtained biomimetic iron complexes were tested as catalysts for cyclohexane oxidation at mild reaction conditions with hydrogenperoxide as oxidant. This stepwise preparation method allowed the synthesis of materials with varying nature and loading of immobilized amino acids. The observed trends in catalytic activity show that both the type and the surface density of immobilized amino acid have an influence on the catalytic performance during cyclohexane oxidation and also on the structure of the immobilized Fe complex.

Introduction

Enzymes catalyze reactions under mild conditions with extremely high selectivity. Manmade catalysts rarely achieve equally good results. It is therefore not surprising that an intensive research activity is going on in the field of biomimetic catalysis. Research takes nature as an example for the design of synthetic catalysts. Metalloenzymes are a particularly interesting design target. They catalyze, among others, a number of highly selective oxidation reactions, using molecular oxygen as the oxidant.[1,2] Classical catalysts fall far behind the metalloenzymes in terms of selectivity and ability to activate molecular oxygen. A prominent example is methane monooxygenase (MMO), which is able to selectively oxidize methane to methanol.[3−5] The active center of MMO is a di-iron core, which is complexed by two histidine and four glutamic acid ligands.[6,7] The two iron atoms are connected via a carboxylate bridge of acetic acid. Copying the active center of MMO in the form of soluble complexes requires considerable synthetic efforts.[8−10] Even if a successful synthesis route has been found, many of the complexes are not stable and tend to dimerize. Moreover, a medium where the biomimetic complex is soluble together with the substrate and the oxidant is often difficult to find.

We therefore chose a different route toward the synthesis of biomimetic catalysts. To eliminate the problems of dimerization and of miscibility/solubility, we decided to immobilize the complex on a support. Moreover, we hypothesized that it is not necessary to synthesize complex ligands that mimic the structure of the enzyme. The amino acids themselves are a simple and truly biomimetic alternative.[11] It was shown that, for example, complexes of copper with various amino acids immobilized in zeolite Y are active oxidation catalysts.[12−15] The preformed Cu complexes were immobilized either by confinement in the supercage of Y zeolite[12,14,15] or by intercalation between the layers of a layered clay mineral. These noncovalent immobilization methods allow the immobilization of well-defined metal amino acid complexes, but the immobilization is reversible in that case and the size of the immobilized complex is limited.

Our strategy was to self-assemble a complex between iron and amino acids by interaction of Fe cations in solution with immobilized amino acid ligands.[16] Amino acids can be covalently anchored on a support by the formation of very stable amide bonds with surface bound amine groups. Amine-functionalized materials are prepared by several methods, for example, by grafting the commercially available 3-aminopropyl-trimethoxysilane on silica surfaces or by co-condensation of the same trimethoxysilane with a silica precursor (typically TEOS).[17−20] If mesoporous silica supports are used

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for surface functionalization, the hydrophobic character inside the pores can be adjusted by co-functionalization with other species.[21] The molar ratio between the two functional groups is adjusted by varying the ratio of the respective silanes during the functionalization reaction. Thereby, well-defined mesoporous materials with a decreasing density of 3-aminopropyl and simultaneously an increasing hydrophobic character inside the pores are obtained. The potential to vary the hydrophobic character of the local environment of an immobilized metal center may be a very important advantage over homogeneous systems because it is well known that the hydrophobic local environment around the active site of enzymes is very important for their catalytic activity.[22] Instead of immobilizing amino acids via the formation of amide bonds with amine-functionalized surfaces, as it was done here, covalent immobilization of amino acids can also be achieved on 3-cloropropyl-functionalized surfaces.[16] The single amino acids immobilized in that way were used to complex copper ions and showed some activity in oxidation reactions.

In the present contribution, we describe the immobilization of amino acids (glutamic acid, histidine, and mixtures thereof) on the above-mentioned co-functionalized porous silica supports, using the automated protocol of a peptide synthesizer. The materials were used to complex iron ions, and the catalytic properties of the metal complexes were evaluated. The work is a first step toward the synthesis of new biomimetic complexes, using a simple procedure that avoids the preparation of synthetic ligands.

Experimental Section

Immobilization of Amino Acids. An amino-functionalized M41S silica material with a narrow pore size distribution in the mesopore range was used as support for immobilization of amino acids. The parent M41S silica has an average BJH pore diameter of 8.0 nm, a specific BET surface area of 1100 m^2/g, and a specific pore volume of 2.6 cm^3/g. Its pore size distribution is very narrow, but the XRD pattern shows only one broad reflection at low 2Θ values; that is, there is no long-range order in the material. Experimental details of the synthesis and a thorough characterization of this material were presented elsewhere.[23] The use of a large pore silica support gives ample space for the immobilization of amino acids and the subsequent formation of metal complexes. The described methodology is, however, not specific for this silica material. It can also be performed with other mesoporous silica materials or commercially available silica products. The silica surface was functionalized with mixtures of 3-aminopropyl and methyl groups, as described in detail in ref 21. By varying the ratio of (3-aminopropyl)trimethoxysilane (APTMS) and methyl-trimethoxysilane (MTMS) in the functionalization reaction mixture, the following ratios of 3-aminopropyl/methyl groups on the surface of the M41S support were obtained: 1:1, 1:2, 1:6, 1:10, 1:20. These ratios are used hereafter to identify the support materials.

Table 1. Pore Characteristics and Carbon and Nitrogen Content of Functionalized M41S Materials with Immobilized Amino Acids

material[a]	pore vol.: [cm^3/g]	surf. area: [m^2/g]	C incr.[b] [wt %]	N incr.[b] [wt %]
His on 1:1	1.06	472	2.4	1.2
His on 1:2	1.14	483	2.5	1.5
His on 1:6	1.29	570	0.9	1.4
His on 1:10	1.51	649	−0.6	0.8
His on 1:20	1.51	690	−1.7	0.4
Glu on 1:1	1.07	470	2.0	0.3
Glu on 1:2	1.19	509	1.8	0.5
Glu on 1:6	1.32	591	0.4	0.6
Glu on 1:10	1.42	587	−0.8	0.2
Glu on 1:20	1.35	607	−2.3	0.2
Glu−His on 1:1[c]	0.89	436	3.8	1.6
Glu+His on 1:1[d]	1.09	478	2.4	1.2

[a] Code, for example, Glu on 1:1: glutamic acid immobilized on functionalized material with 3-aminopropyl/methyl ratio of 1:1. [b] Increase observed during immobilization of amino acids: wt % after immobilization − wt % before immobilization (values of starting materials given in ref 21). [c] Immobilized in the given sequence: (1) Glu, (2) His. [d] Treated with a 1:1 mixture of glutamic acid and histidine during the same cycle.

The immobilization of amino acids was performed by solid-phase peptide synthesis, using a ABI 433 A peptide synthesizer (Applied Biosystems)[24] and standard HBTU/HOBt/NMP activation protocols for Fmoc chemistry (FastMoc protocol, Applied Biosystems).[25] Amino acid side chains were protected as Glu(OtBu) (glutamic acid (*tert*-butyl) ester) and His(Trt) (trityl-histidine). After drying the materials with immobilized amino acids, we deprotected the side chains by shaking in trifluoroacetic acid (20 mL per g of solid) for 30 min in the presence of scavengers (water 5% (v/v), triisopropylsilane 1% (v/v)). The solid was collected by filtration with a glass frit. After deprotection, the solid was shaken for 15 min in a pyridine solution (20% (v/v) in ethanol) and collected by filtration. This washing cycle was repeated five times. Finally, the same cycle was performed two times with pure ethanol. The deprotected, washed solid was dried in ambient atmosphere. Codes identifying the functionalized starting material and the immobilized amino acids are included in Table 1.

The M41S support, a functionalized material with a 3-aminopropyl/methyl ratio of 1:1 ("Funct. 1:1"), and all materials with immobilized amino acids on their surface were treated with Fe(II) in aqueous solution. For that purpose, about 200 mg of solid material was dispersed in 20 mL of demineralized water in a flask. The dispersion was degassed for 5 min with simultaneous vigorous stirring and bubbling N_2. 200 mg of ammonium−iron(II)−sulfate hexahydrate (Mohr Salt $(NH_4)_2[Fe(SO_4)_2]*6H_2O$) was added. The flask was closed with a septum. The colorless solution was stirred for 60 min. After the exchange, the solid was collected by filtration and dried in air.

Characterization. N_2 physisorption measurements were performed at liquid nitrogen temperature with a Micromeritics TriStar 3000 apparatus. Prior to the measurements, the samples were degassed at 673 K and 10 Pa for at least 4 h. The total pore volume was calculated from the volume of adsorbed nitrogen at a relative pressure of $p/p_0 = 0.97$. The surface area was determined from the adsorption branch of the isotherm according to the BET method in the relative pressure range $0.02 < p/p_0 < 0.2$. Organic elementary analysis (CHN) was performed on a LECO CHN-900 apparatus. The organic material in a sample of 2 mg of functionalized M41S was combusted at 1273 K in a flow of He with O_2, and the amounts of evolving CO_2 and N_2 were used to calculate the content of C

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and N. Each measurement was repeated twice. The iron content was determined with a Varian SpectrAA 220 FS AAS. For the measurement, the iron containing solids were dissolved in HF solution. FT-IR spectra were recorded on a Bio-Rad Excalibur FTS 3000 IR spectrometer equipped with an MCT detector at a resolution of 4 cm^{-1}. Samples were measured as self-supporting pellets after 1 h treatment at 473 K in a flow of He in a stainless steel cell equipped with CaF_2 windows. The treatment was performed to remove adsorbed water. For comparison of peak intensities, the spectra were normalized to an identical intensity of the Si−O−Si overtone at 1860 cm^{-1}. Diffuse reflectance UV−vis spectra were measured on a Cary 400 UV−vis spectrometer equipped with a Praying Mantis sample stage from Harrick. The magnetic suszeptibilty χ_m was determined with a magnetometer (PPMS, Quantum Design). The magnetic moment was calculated by fitting of the experimental data with the Curie Law, $\chi_m = C/T$, in the range of 10−300 K. EXAFS spectra at the Fe K-edge were measured at the Swiss-Norwegian Beamline ESRF Grenoble. The beamline uses a Si(111) crystal for monochromizing the X-rays and a Cr mirror to reject harmonics and other high-order reflections. Spectra were collected in fluorescence mode, using a 13-element solid-state Ge detector.

Oxidation of Cyclohexane. All Fe-exchanged materials were tested as catalyst for cyclohexane oxidation with H_2O_2 as oxidant. The oxidation reactions were carried out in 10 mL glass flasks that were sealed with glass lids. Four milliliters of CH_3CN were placed in the flask and degassed with bubbling N_2 for 2 min. Next, 100 μmol of cyclohexane and 110 μmol of H_2O_2 (35% in water) were added. The reaction was started by adding 50 mg of catalyst. The reaction mixture was degassed with N_2 for another 30 s to remove dissolved O_2. The flasks were closed and stirred for 24 h in an oil bath at 303 K. After that, the reaction mixture was analyzed with gas chromatography (HP 5890 Series II, HP-1 column with a FID detector). 1-Pentanol was added as internal standard for quantification. Peaks were assigned with the addition method. The degree of H_2O_2 decomposition was checked by titration with $KMnO_4$.

Results and Discussion

Immobilization of the Amino Acids. The porous 3-aminopropyl/methyl-functionalized M41S materials have several advantages over conventional silica supports carrying only amino groups on their surface. First, the concave pore confines the ligands to a limited space, which is an advantage in the self-assembly of the complexes with iron. Second, mixing the amino groups on the surface with methyl groups allows varying the density of the amino groups on the surface and thereby the density of immobilized amino acids. Moreover, the silica materials become more hydrophobic and therefore more stable against hydrolysis.

Histidine and glutamic acid were immobilized on functionalized materials with molar 3-aminopropyl/methyl group ratios of 1:1, 1:2, 1:6, 1:10, and 1:20. Pore characteristics derived from N_2 physisorption of all of these materials are compiled in Table 1. All materials preserved high pore volumes and specific BET surface areas after immobilization of histidine, glutamic acid, and combinations thereof. The materials with higher surface loading of 3-aminopropyl lose more pore volume because more amino acid is immobilized on the surface. The increase of the carbon and nitrogen content is listed in Tables 1 and 2. The nitrogen loading of all materials increased upon immobilization of amino acids. The increase is larger for histidine, which has three nitrogen atoms as compared to only one in the case of glutamic acid. Within a series of histidine or glutamic acid immobilized on M41S with different 3-aminopropyl loadings, the largest increase of nitrogen content during immobilization is observed for the 1:2 and 1:6 starting materials. The concentration of immobilized amino acid can be calculated from the increase in nitrogen content. These numbers are plotted against the initial concentration of surface amino groups on the silica support in Figure 1. The concentration of immobilized amino acids first increases linearly with a slope of ∼0.6, but reaches a maximum and decreases at high concentrations of surface amines. The reason for the decrease at high amino loadings is that adjacent amino groups start to interact via H-bonding. Moreover, protonation of the amine functionality by unreacted silanol groups on the surface of the silica can occur.[26] Both effects decrease the reactivity of the surface amines toward amino acids. Diluting with methyl groups isolates the amino groups on the surface and caps residual silanol groups. It therefore reduces the extent of protonation and of H-bonding[21] and makes the amino groups more reactive toward the amino acids.

Table 2. Changes of Molar C + N Loadings, Pore Volumes, and Fe Loadings after Immobilization of Amino Acids and Exchange with Fe(II)

material	C incr.[a] [mmol/g]	N incr.[b] [mmol/g]	pore vol. [cm^3/g]	Fe [wt %]	Fe [mmol/g]
M41S support			0.82	0.2	0.04
Funct. 1:1[c]			0.83	3.1	0.55
His on 1:1	2.4	1.0	1.06	0.8	0.14
His on 1:2	2.5	1.3	1.19	0.3	0.05
His on 1:6	0.9	1.2	1.32	0.2	0.04
His on 1:10	−0.5	0.6	1.43	0.2	0.04
His on 1:20	−1.5	0.3	1.5	0.2	0.04
Glu on 1:1	2.0	0.3	1.09	0.5	0.09
Glu on 1:2	1.8	0.4	1.22	0.1	0.02
Glu on 1:6	0.4	0.5	1.35	0.1	0.02
Glu on 1:10	−0.7	0.2	1.41	0.1	0.02
Glu on 1:20	−2.0	0.2	1.29	0.1	0.02
Glu−His on 1:1	4.0	1.4	0.93	1.3	0.23
Glu+His on 1:1	2.4	1.0	1.09	0.5	0.09

[a] Molar C loading normalized to the weight of the silica support: (wt % C incr. * 10/12)/((100 − (wt % C + wt % H + wt % N)/100). [b] Molar N loading normalized to the weight of the silica support: (wt % N incr. * 10/14)/((100 − (wt % C + wt % H + wt % N)/100). [c] M41S material functionalized with 3-aminopropyl/methyl 1:1, treated with iron for comparison.

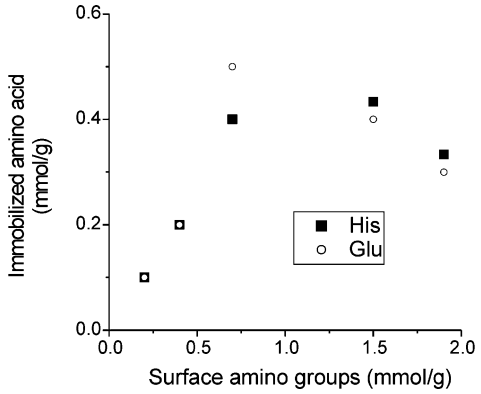

Figure 1. Concentration of immobilized amino acid (Glu or His) versus initial concentration of surface amino groups of the silica support.

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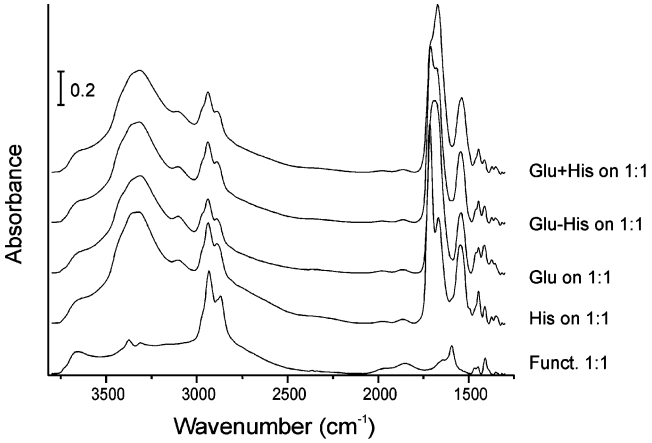

Figure 2. IR spectra of immobilized amino acids. From bottom to top: Funct. 1:1 (M41S with 3-aminopropyl/methyl 1:1), His on 1:1 (histidine immobilized on Funct. 1:1), Glu on 1:1, Glu−His on 1:1 (sequence of Glu and His), Glu+His on 1:1 (mixture of Glu and His). Spectra are offset for better visibility.

The carbon content shows a pattern similar to that of the nitrogen content (see Table 1), but an additional effect is observed. In the materials with small molar loadings of 3-aminopropyl, the carbon content decreases during immobilization of amino acids. The reason is that unreacted methoxy groups of methyl-trimethoxysilane functionalization agent are hydrolyzed during the immobilization procedure and removed from the material.

IR spectroscopy was used to characterize the immobilized amino acids on the silica surface. Upon immobilization of histidine or of glutamic acid, the N−H deformation vibration at 1595 cm^{-1} of the free amine decreases (Figure 2). At the same time, the two characteristic bands for amide groups appear around 1670 and 1550 cm^{-1}. The spectra confirm that the free amine reacted with the amino acid and formed the amide. The very broad bands above 3000 cm^{-1} are characteristic for zwitterionic amino acids.[27] The materials with immobilized amino acids also show an intense band around 1700 cm^{-1}, which is characteristic of carboxylic acid groups. On the materials with immobilized glutamic acid, this band is (partly) caused by the carboxylic acid of the side chain. In the case of immobilized histidine, however, this band must come from the trifluoroacetic acid used in the deprotection step. Also, the material with immobilized glutamic acid contains some trifluoroacetic acid. As a consequence, the carboxylic acid band in the IR spectrum is not well resolved and more intense. When a sequence of glutamic acid and histidine (Glu−His seq. in Figure 2) or a mixture of the two amino acids is immobilized (Glu+His mix. in Figure 2), the spectra show a combination of the features of amide-bound histidine and glutamic acid.

The immobilization of amino acids in mesoporous silica has been described before, but an entirely different approach was applied. Silane-modified peptides were co-condensed with tetraethoxysilane (TEOS) to form a peptide-modified mesoporous silica in one step.[28,29] Our method circumvents the synthesis of silane-modified peptides. Instead, it uses the standardized procedure of an automated peptide synthesizer. As compared to the co-condensation route, our approach is simpler and more flexible with respect to the choice of the amino acids. The preparation of the silica support and the immobilization of the amino acids can be separately optimized. In the co-condensation route, on the other hand, the choice of the amino acid chain influences the formation of the silica mesophase.[29] An advantage of the co-condensation route is that it excludes the presence of residual surface amino groups. We will see in the next paragraph that these play an undesirable role in the complexation of iron.

Complexation of Iron. The immobilized amino acids were used to complex Fe(II) in aqueous solution. The porosity of amino acid-functionalized materials was not affected by the ion exchange with Fe(II) salt (Table 2). This is noteworthy because mesoporous silica materials often suffer from instability toward hydrolysis in aqueous environment. Table 2 shows that histidine is a better ligand for Fe(II) than glutamic acid: Materials with immobilized histidine generally had larger iron loadings than materials with glutamic acid. Interestingly, the highest iron loading is not obtained for the highest amino acid loading (Table 2). The iron loading increases with increasing 3-aminopropyl/methyl ratios. The reason must be that the complexation involves both the amino acids and the residual surface amine groups. Their concentration increases in a series of materials from, for example, His on 1:20 to His on 1:1 (see above). The material with a sequence of histidine and glutamic acid (Glu−His on 1:1) gave the highest iron loading; that is, the dipeptide can complex Fe(II) better than the single amino acids. For comparison, the mesoporous silica support (M41S) was also exchanged with Fe(II) salt. The resulting material had a very low iron content, and it suffered a severe collapse of its mesoporous structure. The amino-functionalized material ("Funct. 1:1" in Table 2) could complex a high concentration of iron, but mainly in the form of large iron oxide clusters. The color of the sample was brownish. Iron oxide clusters were formed because a suspension of "Funct. 1:1" in aqueous solution is strongly alkaline (pH = 10). Under these conditions, insoluble iron hydroxide clusters are very likely to form. The pH of suspensions of materials with immobilized Glu and His is significantly lower (pH ≈ 6.5).

For characterizing the complexes by IR spectroscopy, we chose His on 1:6 and Glu on 1:6 because we presumed that the surface amino groups contribute less to the complexation in these materials than in the materials His/Glu on 1:1. The infrared spectra of the Fe complexes (Figure 3) show some interesting differences between glutamic acid and histidine. In case of histidine, the N−H stretching bands are broadened and shifted to lower wavenumbers by more than 25 cm^{-1} after the iron treatment. That indicates strong electron donation of the lone pair of the amine group to the iron cation. The amine groups are ligands of the complexed iron in the case of immobilized histidine. A second change concerns the region between 1800 and 1450 cm^{-1}. The band at 1720 cm^{-1}, which was assigned to the carbonyl group of

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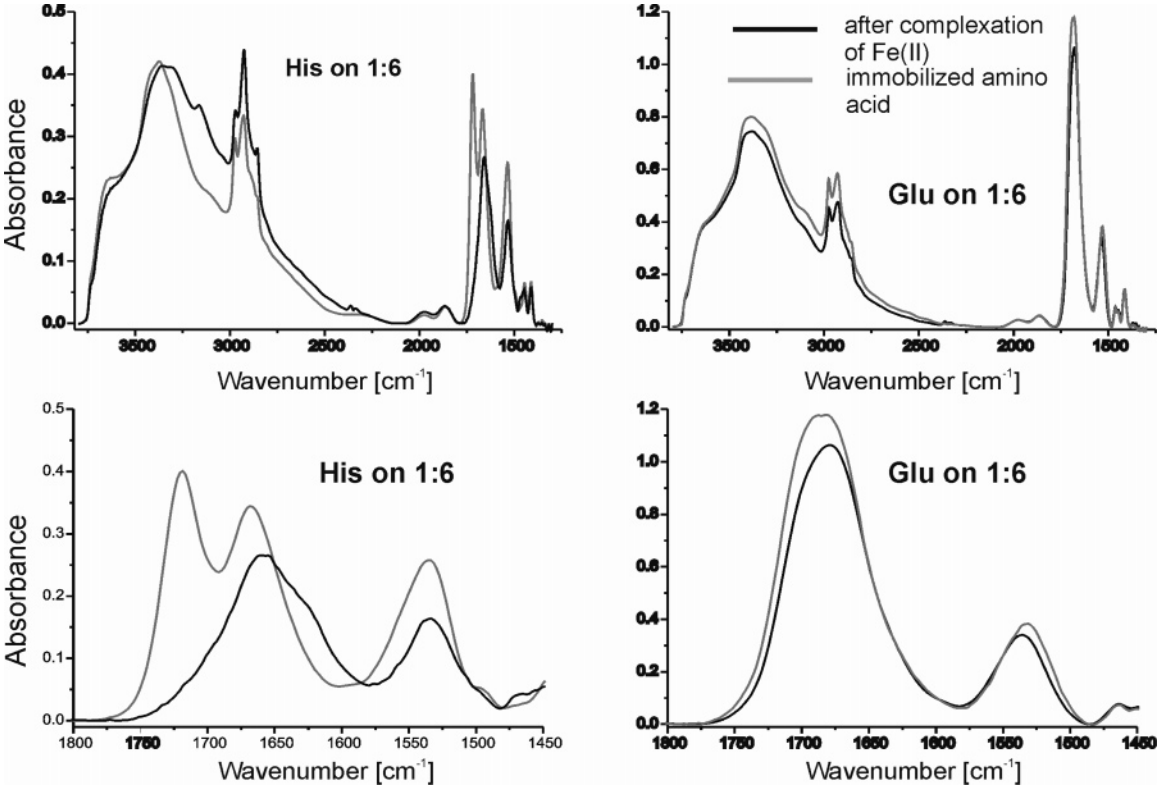
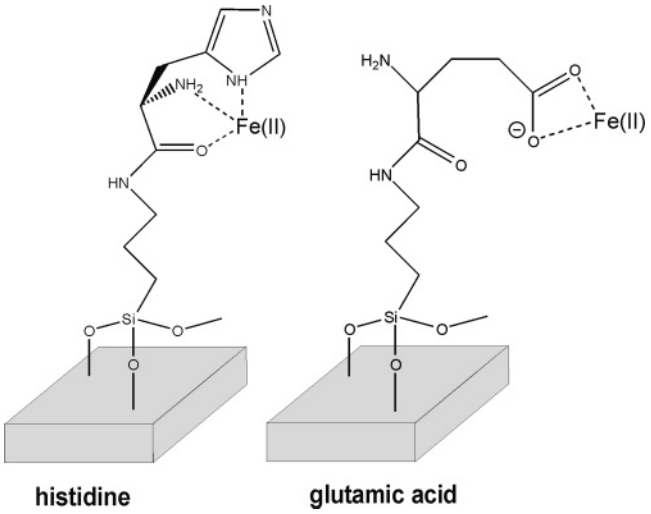


Figure 3. IR spectra of immobilized amino acids before and after treatment with Fe. Histidine (His on 1:6), glutamic acid (Glu on 1:6).

Scheme 1. Possible Modes of Iron Complexation in Materials with Immobilized Histidine and Glutamic Acid (Derived from IR)



the trifluoroacetic acid, disappears. This is due to the removal of this trifluoroacetic acid during the ion exchange in aqueous solution. The C═O stretching band is shifted to lower wavenumbers by 10 cm^{-1}, and in addition a shoulder at even lower wavenumbers appears. The CHN vibration does not shift. This indicates that the lone pairs of the C═O group of the amide act as ligands in the complexation of the iron cations. A possible mode of complexation of the material with immobilized histidine is depicted in Scheme 1. The shoulder of the C═O stretching band could be explained with a fraction of Fe(II) that is not complexed with the amine of the imidazole ring (as depicted in Scheme 1) but only with the free amine of the amino acid. As mentioned before, the other ligands of Fe(II) are neighboring amines on the surface, water, and/or the sulfate counterions used in the ion exchange (not depicted in Scheme 1 for clarity).

The spectra of the materials with immobilized glutamic acid are less affected by the complexation with iron. The stretching vibrations of the amine group are not shifted at all. It can be concluded that the amine groups in this material are hardly involved in iron complexation. Also, the 1800−1450 cm^{-1} range shows less change. The C═O band of trifluoroacetic acid disappears. The overlaid C═O bands of the carboxylate group of the side chain and the amide are both slightly shifted to lower wavenumbers. The CHN vibration of the amide is shifted to larger wavenumbers by 3 cm^{-1}. This indicates that the carboxylate groups of the carboxylic acid side chain and of the amide group are involved in the complexation of the iron cations in that case. A possible iron complexation mode of the material with immobilized glutamic acid is depicted in Scheme 1. The structures proposed in Scheme 1 are in line with the changes observed in the IR spectra before and after iron exchange, but it is clear that the system with immobilized ligands is heterogeneous and more than one complex structure may be present.

UV spectra give information about the environment of the iron complex with immobilized amino acids. The comparison of the UV spectra before and after Fe treatment (Figure 4) shows that two bands at 40 000 and 44 500 cm^{-1} appear after the iron treatment. Both are ligand-to-metal charge-transfer transitions. The strong absorption at higher energies is due to the organic ligands. The iron complexation of the material with histidine shows an additional interesting feature. The absorption of the organic groups is shifted to higher energies

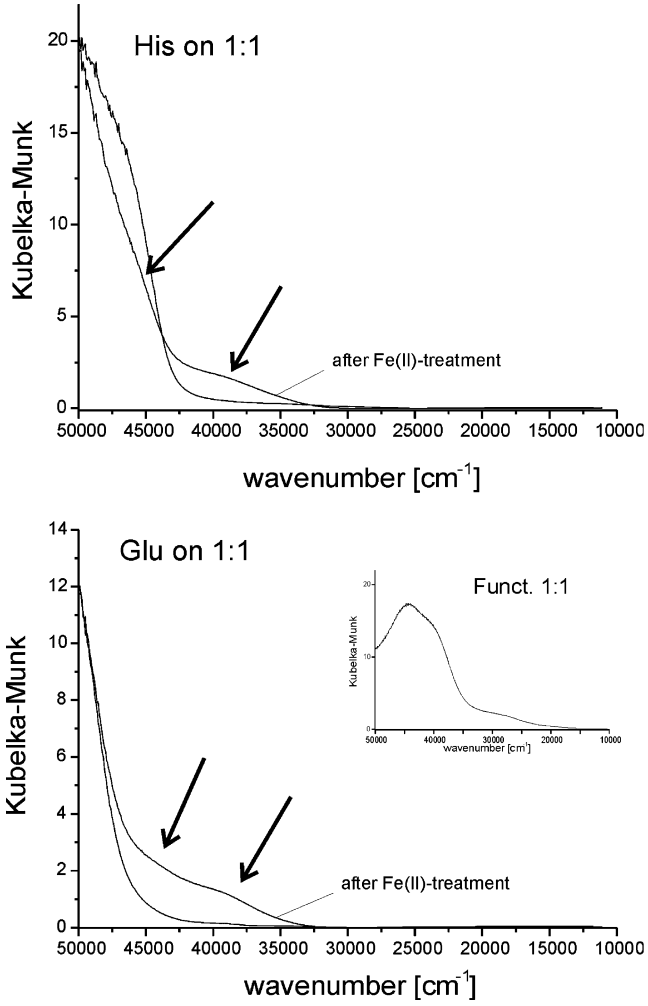


Figure 4. UV spectra of immobilized amino acids before and after treatment with Fe. Histidine (His on 1:1), glutamic acid (Glu on 1:1). Inset: Fe(II) on "Funct. 1:1".

after the Fe treatment. This indicates that the aromatic imidazole moiety donates electron density to the metal, as was postulated on the basis of IR. The UV spectra of the Fe−amino acid complexes differ fundamentally from the species formed on the amine-functionalized support "Funct. 1:1", which does not carry immobilized amino acids (inset of Figure 4). Latter material shows bands at 20 000 and 30 000 cm^{-1} that are characteristic of clustered iron species.[30]

An important question is whether iron is complexed in the form of isolated ions or in the form of oxo- or hydroxo-bridged dimers. The EXAFS spectra of dehydrated "His on 1:1" and of "Glu on 1:1" show two maxima in the Fourier transform (Figure 5). The first one is due to the oxygen and nitrogen atoms in the first coordination shell of iron (total coordination number ≈ 3). The second, more distant shell can be fitted by an iron neighbor (which would imply the presence of an iron dimer), but also by a C/N atom of the amino acid ligand or by a sulfur atom, originating from the sulfate anion used in the ion exchange. Because of the limited data range, it is not possible to discriminate between these options. EXAFS, therefore, does not give any conclusive evidence in favor of either a mononuclear or a dinuclear

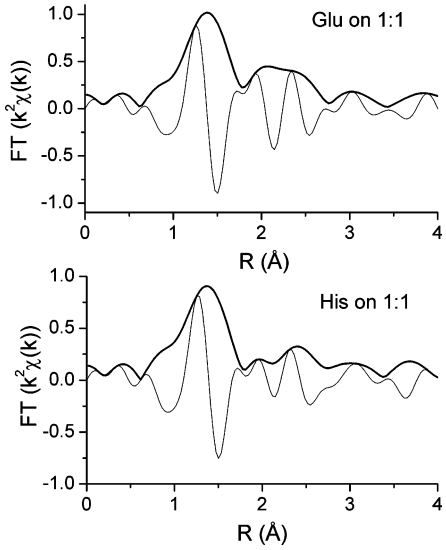


Figure 5. Fourier transform (magnitude and imaginary part) of the EXAFS spectra $k^2*\chi(k)$ of Glu on 1:1 and His on 1:1 after treatment with Fe(II). A Hanning window function was applied in the interval of 2.7−10.6 $Å^{-1}$.

complex. The XANES and pre-edge features of the spectra indicate that iron is partly oxidized to Fe(III). It is well known that hydrated Fe(II) readily oxidizes to Fe(III) in the presence of air. The intensity of the pre-edge of the dehydrated samples is between the values expected for a tetrahedral and octahedral coordination.

The magnetization curve of Fe-exchanged "His on 1:1" as a function of temperature obeys essentially the Curie law and can be fitted with an effective paramagnetic moment of $\mu/\beta = 4.75 \pm 0.2$. This is close to the expected value for isolated high-spin Fe^{2+} ions and suggests that the majority of the iron centers are mononuclear.

We can compare our results with the known structures of Cu^{2+} amino acid complexes.[31] Amino acids without coordinating groups in the side chain complex Cu^{2+} via the oxygen of the carboxyl group and the nitrogen of the α-amino group, provided that the pH is sufficiently high. With histidine, the basic nitrogen atoms of the imidazole ring can replace the carboxylate group or act as an additional ligand.[14] In our materials, the terminal carboxylate group of the amino acid is converted to an amide, which is a weaker ligand. Instead, the imidazole ring of histidine and the carboxylic acid group of glutamic acid contribute to the complexation of iron (Scheme 1). Our results confirm earlier experience that histidine forms especially stable complexes. Note that histidine is part of the coordination sphere of most non-heme iron enzymes.

Catalytic Tests. Cyclohexane oxidation with H_2O_2 was performed to evaluate the catalytic properties of the samples. All immobilized iron complexes had reasonable activities at the reaction temperature of 303 K. A blind test without catalyst gave no reaction products and no oxidant decomposition. None of the Fe containing materials decomposed H_2O_2 completely after 24 h of reaction. Although no leaching of iron into the solution could be detected, the materials had a lower activity when reused.

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Table 3. Cyclohexane Oxidation Activity of Fe-Exchanged Materials with Immobilized Amino Acids (24 h Reaction Time)

		TOF		
Fe material	tot. prod.[a] [μmol]	CyOL[b] [10^{-6} s^{-1}]	CyON[c] [10^{-6} s^{-1}]	sel.[d] [% CyOL]
no catalyst				
M41S support	0.96	1.58	4.04	28
Funct. 1:1	3.28	0.56	0.89	39
His on 1:1	0.92	0.79	1.34	37
His on 1:2	0.62	0.90	1.70	35
His on 1:6	0.46	0.88	1.99	31
His on 1:10	0.08	0.07	0.46	13
His on 1:20	0.07	0.04	0.41	10
Glu on 1:1	0.94	0.66	1.50	31
Glu on 1:1[e]	0.50	0.17	1.1	13
Glu on 1:2	0.22	0.73	2.18	25
Glu on 1:6	0.01	0	0.11	0
Glu on 1:10	0.13	0.33	1.38	19
Glu on 1:20	0.69	2.25	6.55	26
Glu−His on 1:1	1.01	0.35	0.64	35
Glu+His on 1:1	0.85	0.74	1.53	33

[a] Total amount of formed products: μmol of cyclohexanol + μmol of cyclohexanone. [b] Turnover frequency of cyclohexanol: TOF CyOL = mol of cyclohexanol/(mol of Fe * s reaction time). [c] Turnover frequency of cyclohexanone: TOF CyON = mol of cyclohexanone/(mol of Fe * s reaction time). [d] Selectivity toward cyclohexanol: sel = μmol of cyclohexanol/(μmol of cyclohexanol + μmol of cyclohexanone). [e] Catalyst recycled and reused under identical reaction conditions.

A comparison of TOF values for the formation of cyclohexanol and cyclohexanone (Table 3) shows that no general trend between the amount of Fe and the activity of the catalyst can be made. For the materials with immobilized histidine, the TOF values are constant for materials with high loadings of immobilized amino acids (His on 1:1, 1:2, and 1:6 in Table 3) and decrease rapidly as soon as the density of immobilized amino acids goes down. For the materials with immobilized glutamic acid, it is exactly the opposite. The highest TOF was obtained for the material with the lowest amount of immobilized glutamic acid (Glu on 1:20 in Table 3). It seems that a higher concentration of immobilized histidine on the surface is advantageous for the catalytic activity, whereas it is the opposite with immobilized glutamic acid. The chemical environment, the nature of the ligands and the proximity between two active sites, influences the activity. The low selectivity toward the alcohol indicates, however, that the reaction mechanism involves free radical reactions and is not biomimetic. That is confirmed by the rather high activity of the iron complexes with the support material (Funct. 1:1) containing no immobilized acids.

Conclusions

The use of an automated peptide synthesizer for anchoring amino acids on an amino-functionalized silica provides a simple and versatile method for immobilizing amino acids on a porous support. The immobilized amino acids form stable complexes with iron, which have moderate activity in the oxidation of cyclohexane. The nature of the immobilized amino acid and its surface density has an influence on the catalytic activity. Selectivity and activity of the complexes are lower than those of the metalloenzymes they are supposed to mimic. This is not surprising. The complexes of amino acids with iron are very simple. They involve only one or two amino acids plus amine groups from the support. In contrast to most non-heme iron enzymes, they are mono- and not binuclear. To improve the stability and catalytic performance of the iron complexes, it will be necessary to use longer peptide chains that can form multidentate ligands without interference of surface bound amino groups and also to optimize the distance between two peptide chains on the surface so as to promote the formation of dimers. There is a lot of room for improvement, but the first results are encouraging and show that the method has potential for the synthesis of new biomimetic catalysts.

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